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Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry

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Abstract: Flavonol glycoside composition and content in blueberry and blackberry extracts were determined using a high-performance liquid chromatographic (HPLC) separation method coupled with photodiode array (PDA) and mass spectrometric (MS) detection. The hydrophilic antioxidant capacities of crude and fractionated flavonol extracts were also determined by the oxygen radical-absorbing capacity ($ORAC_{FL}$) and photochemiluminescence (PCL) assays. Eight flavonols of quercetin and quercetin–sugar conjugates were identified in Kiowa blackberry, namely rutinoside, galactoside, methoxyhexoside, glucoside, pentoside, [6″-(3-hydroxy-3-methylglutaroyl)]-β-galactoside, glucosylpentoside and oxalylpentoside. Thirteen flavonols were detected in Ozarkblue blueberry. Of these, myricetin 3-hexoside and 12 quercetin–sugar conjugates, namely rutinoside, galactoside, methoxyhexoside, glucoside, pentoside, glucosylpentoside, caffeoylglucoside, oxalylpentoside, rhamnoside, dimethoxyrhamnoside, acetylgalactoside and acetylglucoside, were identified. In Bluecrop blueberry, two additional quercetin–sugar conjugates were identified, namely glucuronide and caffeoylgalactoside. Quercetin glycosides accounted for 75% of total flavonols in the blueberry genotypes. Total flavonol contents ranged from 99 to 150 mg kg^{-1} for blackberries and from 192 to 320 mg kg^{-1} for blueberries. Quenching of peroxyl and superoxide anion radicals by the flavonol fractions ranged from 1.5 to 2.3 mmol Trolox equivalents (TE) kg^{-1} and from 0.5 to 0.7 mmol TE kg^{-1} respectively for blackberries and from 2.9 to 5.2 mmol TE kg^{-1} and from 0.8 to 1.4 mmol TE kg^{-1} respectively for blueberries. The HPLC method allowed for complete separation and identification of flavonols commonly found in blackberries, and blueberries. Our results showed that blueberry and blackberry genotypes varied significantly in flavonol content and antioxidant capacity. Even though total flavonol content did not correlate well with antioxidant capacity, their ability to scavenge peroxyl and superoxide anion radicals was apparent.

Keywords: flavonol glycoside; antioxident capacity; $ORAC_{FL}$; PCL; blackberry; blueberry; HPLC/MS

INTRODUCTION

Blackberries and blueberries are known to contain appreciable levels of phenolic compounds, including anthocyanins, flavonols, chlorogenic acid and procyanidins, that have high biological activity and may provide health benefits as dietary antioxidants.[1–9] Flavonoids are potent antioxidants and some studies suggest that high flavonoid intake is protective against many chronic diseases, including coronary heart disease, certain cancers and other degenerative diseases that are linked to oxidative stress.[10–13] Flavonoids from berries are thought to play an important role in protection against oxidative damage in biological systems owing to their ability to scavenge various free radical species. Two commonly used methods to measure free radical-scavenging activities of foods are the oxygen radical-absorbing capacity ($ORAC_{FL}$)[14] and photochemiluminescence (PCL)[15] assays. Since phytochemical antioxidants differ in their ability to scavenge different free radicals, multiple assays using different biologically relevant radical sources may be important to fully understanding the antioxidant capacity of a sample.[3,16]

Although anthocyanin glycosides have been well characterised in berries,[8,9,17] little information exists on the composition and content of flavonol glycosides

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in the fruit owing to difficulty in separation and lack of commercial standards. Improved separation of flavonoid glycosides was reported for apple pomace and juice and extracts of pear fruits of different cultivars using a polar end-capped C_{18} column,[18,19] but the methodology has not been tested on blueberries and blackberries which contain a variety of quercetin conjugates.

In our previous work,[8] flavonol glycosides in berries were not readily separated on a conventional reverse phase C_{18} column, which limited their identification and quantification. This study was undertaken to establish a high-performance liquid chromatographic (HPLC) method coupled with photodiode array (PDA) and mass spectrometric (MS) detection that could be used to effectively separate, identify and quantify flavonol glycosides in blueberry and blackberry genotypes. The antioxidant capacities of fractionated flavonol extracts against peroxyl ($ROO^{\bullet}$) and superoxide anion ($O_2^{\bullet -}$) radicals were also determined using $ORAC_{FL}$ and PCL methods.

EXPERIMENTAL

Chemicals

Quercetin 3-glucoside and quercetin 3-galactoside were obtained from Indofine Chemical Company (Somerville, NJ, USA), rutin, fluorescein and gallic acid from Sigma Chemical Company (St Louis, MO, USA), HPLC-grade methanol and acetonitrile from JT Baker Inc (Phillipsburg, NJ, USA), formic and acetic acids from Burdick & Jackson (Muskegon, MI, USA), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) from Wako Chemicals Inc (Richmond, VA, USA) and Trolox (6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid) from Aldrich (Milwaukee, WI, USA).

Samples

Mature berries assessed by full colour development were harvested in 2003 and stored at −20 °C until analysis. Blueberry genotypes analysed included two commercial cultivars, Bluecrop (*Vaccinium corymbosum* L with common name northern highbush type) and Ozarkblue (a hybrid of majority *V corymbosum* germplasm with some contribution from *V darrowi* L and *V ashei* Reade and in the group of blueberries known as southern highbush), and three advanced breeding selections, A-98, US-720 and US-729 (southern highbush hybrids), which are small-fruited genotypes with exceptionally dark skin colour. Selection A-98 is a $\frac{3}{4}$ *V corymbosum* + $\frac{1}{4}$ *V darrowi* hybrid, while US-720 and US-729 have as common parents in their backgrounds the species *V corymbosum, V ashei, V darrowi* and *V atrococcum* Heller and are unique hybrids in blueberry breeding owing to this combination of four species. The six blackberry genotypes analysed were Apache, Arapaho, Chickasaw, Kiowa, Navaho and Prime-Jim® (APF-12). The blackberries are considered as *Rubus* subgenus *Rubus* Watson, as a species classification is not possible owing to their broad genetic background of several native North American species (the major one being *R allegheniensis* Porter) along with some other species that contribute the gene for thornlessness (Apache, Arapaho and Navaho). They are derived largely from the same original parent material and as a group do not vary greatly in overall genetic background.

Analyses

Extraction

Approximately 100 g of frozen berries were blended to a puree, and 5 g subsamples were homogenised for 1 min in 20 ml of extraction solution containing methanol/water/formic acid at a ratio of 60:37:3 by volume. Homogenates were filtered through Miracloth (CalBiochem, LaJolla, CA, USA), and the filtrates were centrifuged for 10 min at $2739 \times g$. Aliquots (4 ml) of supernatant were evaporated to dryness using a SpeedVac® concentrator (ThermoSavant, Holbrook, NY, USA), with no radiant heat applied during concentration, and re-suspended in 1 ml of 20 g kg^{-1} acetic acid solution. All samples were passed through 0.45 μm filters (Whatman, Clifton, NJ, USA) prior to HPLC analysis. Triplicate extractions were prepared from each fruit genotype.

HPLC analysis of flavonols

Samples (50 μl) were analysed using a Waters HPLC system (Waters Corp, Milford, MA, USA) equipped with a model 600 pump, model 717 plus autosampler and model 996 photodiode array detector. Separation was carried out using a 4.6 mm × 250 mm Aqua® C_{18} column (Phenomenex, Torrance, CA, USA) with a 3.0 mm × 4.0 mm ODS® C_{18} guard column (Phenomenex). The mobile phase was a gradient of 20 g kg^{-1} acetic acid (A) and 5 g kg^{-1} acetic acid in water and acetonitrile (50:50 v/v, B) from 10% B to 55% B in 50 min and from 55% B to 100% B in 10 min.[19] The system was equilibrated for 20 min at the initial gradient prior to each injection. A detection wavelength of 360 nm was used for flavonols at a flow rate of 1 ml min^{-1}. Flavonols were expressed as mg rutin equivalents kg^{-1} fresh weight.

Isolation of flavonols by semi-preparative HPLC

Fractionation of flavonols was performed using a preparative Aqua® C_{18} column (10 mm × 250 mm) at a flow rate of 4.7 ml min^{-1} with the same gradient conditions as described above. Flavonol peaks monitored at 360 nm that eluted from 30 to 50 min (Fig 1) were collected using a Waters fraction collector II. The fractions containing the flavonol peaks were pooled and defined as 'fractionated flavonols'. The pooled fraction of flavonols (approximately 28 ml) was evaporated to dryness using a SpeedVac® concentrator and re-suspended in 200 μl of methanol. The fractionated flavonol fractions obtained from each berry genotype were analysed for antioxidant capacity using the $ORAC_{FL}$ and PCL assays.

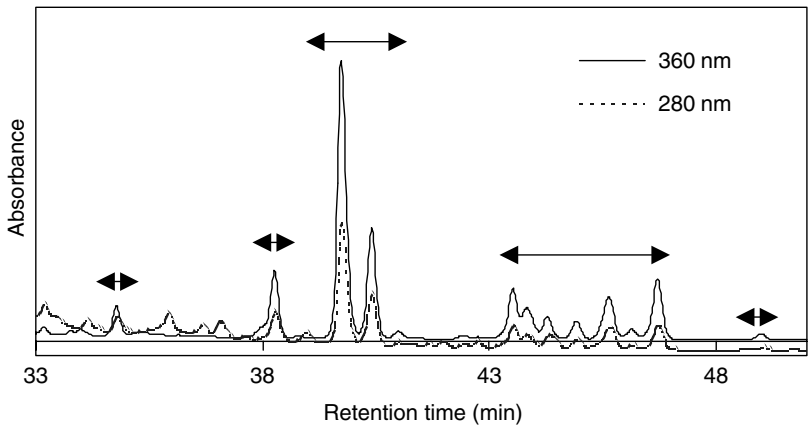

Figure 1. Fractionated region of flavonol glycosides (360 nm) in Bluecrop blueberry. Arrows indicate the peaks collected in the flavonol fraction.

HPLC/ESI-MS analysis of flavonols

An analytical Hewlett Packard 1100 series HPLC instrument (Palo Alto, CA, USA) equipped with an autosampler, binary HPLC pump and UV/VIS detector was used. Flavonols were separated using the same HPLC conditions as described above with detection at 360 nm. For HPLC/MS analysis the HPLC apparatus was interfaced to a Bruker (Billerica, MA, USA) model Esquire-LC/MS ion trap mass spectrometer. Mass spectral data were collected with the Bruker software, which also controlled the instrument and collected the signal at 360 nm. Typical conditions for mass spectral analysis in the negative ion electrospray mode included a capillary voltage of 4000 V, a nebulising pressure of 30.0 psi, a drying gas flow of 9.0 ml min^{-1} and a temperature of 300 °C. Data were collected using the full scan mode over a mass range of m/z 50–1000 at 1.0 s per cycle. Characteristic ions were used for peak assignment (Table 1). For compounds where chemical standards were commercially available, retention times were also used to confirm the identification of components.

Table 1. Peak assignments, retention times (RT) and mass spectral data of flavonol glycosides detected in blackberry and blueberry genotypes

Peak	HPLC RT (min)	Identification	*m/z* M$^-$	*m/z* Fragments
1	34.7	Myricetin 3-hexoside	479	317 [M – hexose], (myricetin)
2	39.1	Quercetin 3-rutinoside	609	463 [M – rhamnose], 301 [463-glucose], (quercetin)
3	40.2	Quercetin 3-galactoside	463	301 [M – galactose], (quercetin)
4	40.4	Quercetin 3-methoxyhexoside	493	463 [M – methoxy], 301 [463-hexose], (quercetin)
5	40.9	Quercetin 3-glucoside	463	301 [M – glucose], (quercetin)
6	42.8	Quercetin 3-pentoside	433	301 [M – pentose], (quercetin)
7	43.1	Quercetin 3-glucuronide	477	301 [M – glucuronic acid], (quercetin)
8	43.3	Quercetin 3-*O*-[6″-(3-hydroxy-3-methylglutaroyl)]-β-galactoside	607	463 [M – hydroxymethylglutaric acid], 301 [463-galactose], (quercetin)
9	43.8	Quercetin 3-glucosylpentoside	595	433 [M – glucose], 301 [433-pentose], (quercetin)
10	44.0	Quercetin 3-caffeoylgalactoside	623	463 [M – caffeic acid], 301 [463-galactose], (quercetin)
11	44.6	Quercetin 3-caffeoylglucoside	623	463 [M – caffeic acid], 301 [463-glucose], (quercetin)
12	45.2	Quercetin 3-oxalylpentoside	505	433 [M – oxalic acid], 301 [433-pentose], (quercetin)
13	45.8	Quercetin 3-rhamnoside	447	301 [M – rhamnose], (quercetin)
14*	46.0	Unidentified	589	567, 447, 315
15	46.3	Quercetin 3-dimethoxyrhamnoside	507	477 [M – methoxy], 447 [477-methoxy], 301 [447-rhamnose], (quercetin)
16	46.8	Quercetin 3-acetylgalactoside	505	463 [M – acetic acid], 301 [463-galactose], (quercetin)
17	48.5	Quercetin 3-acetylglucoside	505	463 [M – acetic acid], 301 [463-glucose], (quercetin)
18	59.0	Quercetin	301	(Quercetin)

Determination of total soluble phenolics

Total soluble phenolics (TPH) in the methanol/formic acid/water extracts were analysed using the Folin–Ciocalteu method,[20] with results expressed as mg gallic acid equivalents kg^{-1} fresh weight.

Determination of antioxidant capacities

The oxygen radical-absorbing capacity ($ORAC_{FL}$) of extracts was determined using the method of Cho *et al*[8] with minor modifications. Fruit extracts were diluted 150-fold (for fractionated flavonols) or 800-fold (for crude extracts) with phosphate buffer (75 mM, pH 7) prior to $ORAC_{FL}$ analysis. Each well had a final volume of 480 µl. A 40 µl aliquot of a working stock solution (99.1 mM) of AAPH was injected into each well. The polynomial regression equations obtained from Trolox equivalent (TE) standard curves were used to calculate final $ORAC_{FL}$ values. The photochemiluminescence (PCL) assay with a Photochem® instrument (Analytik Jena AG, Jena, Germany) was used to measure the hydrophilic antioxidant activity of extracts against superoxide anion radicals generated from luminol, a photosensitiser, when exposed to UV light. The antioxidant activity of berry extracts was measured using an 'ACW' kit provided by the manufacturer designed to measure the antioxidant activity of water-soluble compounds.[21] The antioxidant activity estimated by duration of lag phase was compared with a TE standard curve and expressed as mmol TE kg^{-1} fresh weight.

Statistical analysis

Analysis of variance[22] was used to determine significant differences ($P < 0.05$) in TPH and flavonol contents and antioxidant capacities among genotypes of each fruit analysed. Pearson's correlation test was used to determine the relationships between $ORAC_{FL}$ and PCL values and contents of total phenolics and total flavonols.

RESULTS AND DISCUSSION

HPLC method

The separation of flavonol glycosides in Bluecrop blueberries on a conventional C_{18} reverse phase column (Symmetry®, A) and polar end-capped reverse phase C_{18} column (Aqua®, B) (Waters Corp, Milford, MA, USA) is shown in Fig 2. Near baseline separation of the flavonol glycosides was achieved using the Aqua® column. The flavonols in Bluecrop blueberry were identified as myricetin 3-hexoside (peak 1), quercetin 3-rutinoside (peak 2), quercetin 3-galactoside (peak 3), quercetin 3-methoxyhexoside (peak 4), quercetin 3-glucoside (peak 5), quercetin 3-glucuronide (peak 7), quercetin 3-glucosylpentoside (peak 9), quercetin 3-caffeoylgalactoside (peak 10), quercetin 3-rhamnoside (peak 13), quercetin 3-dimethoxyrhamnoside (peak 15), quercetin 3-acetylgalactoside (peak 16) and quercetin 3-acetylglucoside (peak 17). The Aqua® column readily separated quercetin 3-glucoside (peak 5) and quercetin 3-rutinoside (peak 2), which co-eluted

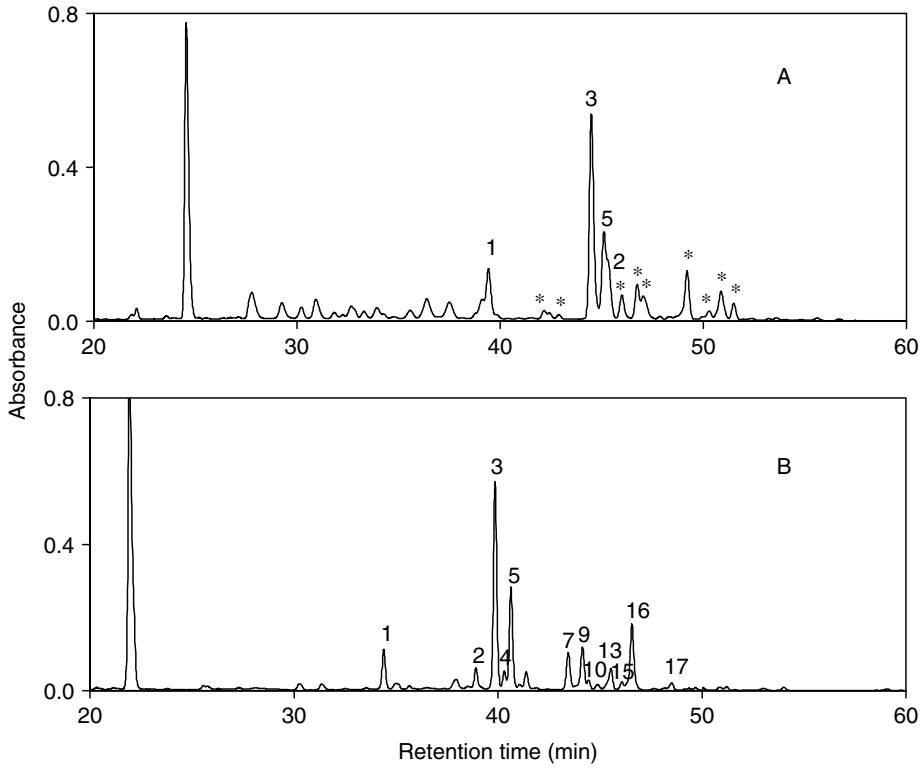

Figure 2. Separation and detection (360 nm) of flavonol glycosides in Bluecrop blueberry on (A) Symmetry® C_{18} and (B) Aqua® C_{18} columns. See Table 1 for peak identification. *Unidentified flavonols. A linear gradient of 50 g kg^{-1} formic acid (A) and methanol (B) from 2% B to 60% B for 60 min at 1 ml min^{-1} was used for the Symmetry® C_{18} column.

on the Symmetry® column, and the polar stationary phase dramatically improved resolution of the flavonol glucosides compared with the Symmetry® column. Using similar HPLC methodology, Schieber *et al*[18,19] obtained excellent separation of quercetin glycosides in apple and pear extracts.

The HPLC profile of flavonols detected at 360 nm in Kiowa blackberry is shown in Fig 3A. The flavonols in Kiowa blackberry were identified as quercetin 3-rutinoside (peak 2), quercetin 3-galactoside (peak 3), quercetin 3-methoxyhexoside (peak 4), quercetin 3-glucoside (peak 5), quercetin 3-pentoside (peak 6), quercetin 3-*O*-[6″-(3-hydroxy-3-methylglutaroyl)]-*β*-galactoside (peak 8), quercetin 3-glucosylpentoside (peak 9), quercetin 3-oxalylpentoside (peak 12), an unidentified peak (peak 14) and quercetin (peak 18). The identification of peaks 2, 3, 5 and 8 was consistent with previous reports,[7,8,23–25] but quercetin 3-glucosylpentoside, quercetin 3-oxalylpentoside and quercetin 3-methoxyhexoside have not previously been identified. Henning[24] previously identified kaempferol 3-glucuronide, kaempferol 3-glucoside, kaempferol 3-galactoside and kaempferol 3-xylosylglucuronide in blackberries, but no kaempferol glycosides were detected in the genotypes we analysed. This discrepancy is most likely due to differences in genetics, since the material we analysed is derived largely from eastern US native germplasm, whereas the cultivars analysed by Henning[24] were derived from European germplasm.

The HPLC profile of flavonols detected at 360 nm in Ozarkblue blueberry is shown in Fig 3B. Thirteen flavonols were detected in Ozarkblue, namely myricetin 3-hexoside (peak 1), quercetin 3-rutinoside (peak 2), quercetin 3-galactoside (peak 3), quercetin 3-methoxyhexoside (peak 4), quercetin 3-glucoside (peak 5), quercetin 3-pentoside (peak 6), quercetin 3-glucosylpentoside (peak 9), quercetin 3-caffeoylglucoside (peak 11), quercetin 3-oxalylpentoside (peak 12), quercetin 3-rhamnoside (peak 13), quercetin 3-dimethoxyrhamnoside (peak 15), quercetin 3-acetylgalactoside (peak 16) and quercetin 3-acetylglucoside (peak 17). Kader *et al*[26] previously identified the main flavonol glycosides in highbush blueberries as quercetin 3-glucoside, quercetin 3-galactoside and quercetin 3-rhamnoside, which concurs with our findings. Although quercetin 3-galactoside and quercetin 3-glucoside have identical *m/z* values, we confirmed that the galactoside eluted before the glucoside using authentic standards of the two compounds, which corroborated findings by Schieber *et al*.[19] In contrast to our results, low levels of kaempferol 3-glucoside were detected in both highbush (Coville)[26] and southern highbush (Sierra)[27] blueberry cultivars.

Flavonols, total phenolics and antioxidant activities in blackberries

The contents of individual flavonols, total flavonols and total phenolics of blackberry genotypes are presented in Table 2. The total flavonol content of blackberry genotypes ranged from a low of 99 mg kg^{-1} for Chickasaw to a high of 150 mg kg^{-1} for Apache, reflecting a 1.5-fold difference among genotypes, while the other four genotypes did not vary markedly in total flavonol content. The abundance of total flavonols and rankings of the six genotypes were similar to those in our previous study (2002 crop), where total flavonols ranged from a low of 102 mg kg^{-1} for Chickasaw to a high of 160 mg kg^{-1} for Apache.[8] The total flavonol values of the blackberry genotypes also agreed well with the values reported by Siriwoharn and Wrolstad[7] for Evergreen (178 mg kg^{-1}) and Marion (116 mg kg^{-1}) blackberries, but were much

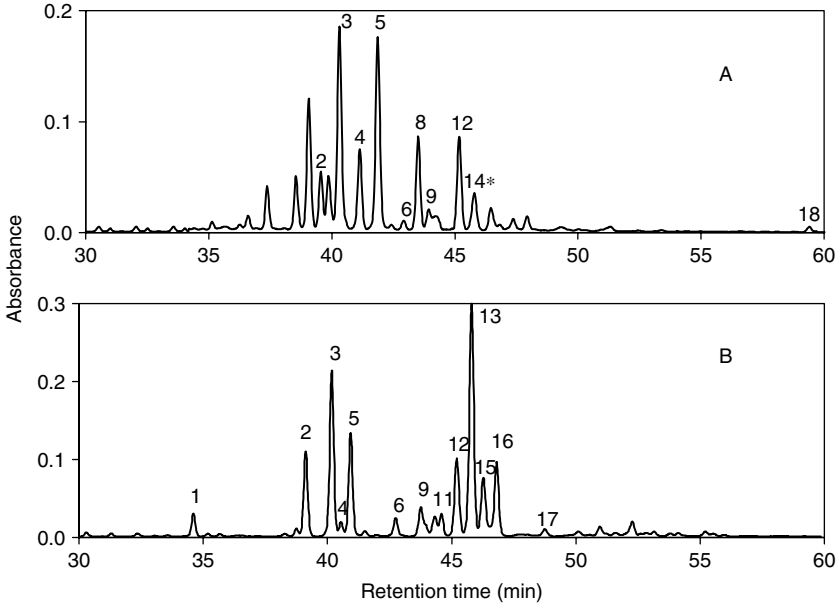

Figure 3. Detection of flavonol glycosides (360 nm) in (A) Kiowa blackberry and (B) Ozarkblue blueberry. See Table 1 for peak identification.
*Unidentified flavonol.

Table 2. Flavonol and total phenolic contents (mg kg^{-1} fresh weight[a]) of blackberry genotypes

Compound	Genotype					
	Apache	Prime-Jim®	Arapaho	Chickasaw	Kiowa	Navaho
Quercetin 3-rutinoside	11.5 ± 0.1[d]	20.2 ± 0.1	12.1 ± 0.1	7.9 ± 0.1	8.0 ± 0.2	7.3 ± 0.1
Quercetin 3-galactoside	49.3 ± 0.2	9.0 ± 0.1	23.7 ± 0.5	18.5 ± 0.4	26.3 ± 0.1	32.6 ± 0.4
Quercetin 3-methoxyhexoside	4.1 ± 0.1	17.2 ± 0.1	7.6 ± 0.1	4.2 ± 0.1	11.1 ± 0.1	9.2 ± 0.1
Quercetin 3-glucoside	24.6 ± 0.1	41.5 ± 0.3	15.1 ± 0.1	39.1 ± 0.5	20.2 ± 1.6	15.4 ± 0.1
Quercetin 3-pentoside	ND	3.6 ± 0.1	2.0 ± 0.1	ND	9.9 ± 1.3	2.5 ± 0.1
Quercetin 3-[6″-(3-hydroxy-3-methyglutaroyl)]-β-galactoside	41.9 ± 0.2	ND	17.2 ± 0.5	14.5 ± 0.3	12.8 ± 0.1	19.5 ± 0.2
Quercetin 3-glucosylpentoside	4.5 ± 0.1	ND	4.2 ± 0.1	ND	3.9 ± 0.1	4.2 ± 0.1
Quercetin 3-oxalylpentoside	5.6 ± 0.1	10.9 ± 0.1	9.1 ± 0.1	8.1 ± 0.2	13.1 ± 0.1	12.6 ± 0.1
Unknown[b]	2.5 ± 0.1	10.8 ± 0.1	4.8 ± 0.1	ND	6.5 ± 0.1	7.0 ± 0.1
Quercetin	6.3 ± 0.1	4.9 ± 0.1	6.3 ± 0.4	6.5 ± 0.1	1.8 ± 0.1	9.6 ± 0.2
Total flavonols[c]	150a	118b	102c	99c	114bc	120b
Total phenolics[c]	4440a	2922e	3756c	4110b	3495d	4464a

[a] Data expressed as rutin equivalents for flavonols and gallic acid equivalents for total phenolics.
[b] Data expressed as rutin equivalents.
[c] Values within a row with similar letters are not significantly different (LSD, $P > 0.05$).
[d] Standard deviation ($n = 3$). ND, not detected.

lower than the range of values (142–435 mg kg^{-1}) reported by Henning[24] for five blackberry cultivars. The TPH content of the blackberry genotypes ranged from a low of 2922 mg kg^{-1} for Prime-Jim® to a high of 4464 mg kg^{-1} for Navaho. The contents of total phenolics were similar to values reported by Heinonen *et al*[2] (4350 mg kg^{-1}), Wada and Ou[23] (4950 mg kg^{-1}), Moyer *et al*[28] (mean 4780 mg kg^{-1}, $n = 32$), Sellappan *et al*[5] (mean 4865 mg kg^{-1}, $n = 2$) and Wang and Lin[29] (mean 2260 mg kg^{-1}, $n = 3$), but much lower than the values reported by Siriwoharn and Wrolstad[7] (mean 8300 mg kg^{-1}, $n = 2$). The levels of total flavonols and total phenolics did not correlate well ($r_{xy} = 0.33$), which is not surprising considering that total flavonols accounted for only 2.4–4.0% of the total soluble phenolics in the blackberry genotypes. Although the total phenolic results are most likely inflated owing to non-phenolic reducing compounds reacting with the Folin–Ciocalteu reagent,[30] the results clearly show that flavonols constituted a minor proportion of total phenolics in blackberries.

Quercetin 3-galactoside was the predominant flavonol in Apache, Arapaho, Kiowa and Navaho, while quercetin 3-glucoside was the predominant flavonol in Prime-Jim® and Chickasaw. Apache contained the highest amount of quercetin 3-*O*-[6″-(3-hydroxy-3-methylglutaroyl)]-β-galactoside, while Prime-Jim® contained none. Myricetin was not detected in any of the blackberry genotypes, which confirmed previous findings.[1,8] Blackberries in the University of Arkansas breeding programme appear to be unique in that they only contain derivatives of quercetin, indicating that they lack the genetic capacity to synthesise the enzyme flavonoid 3′,5′-hydroxylase which converts dihydrokaempferol to dihydromyricetin, the key step in the biosynthetic pathway for myricetin.[31] Several studies have reported the presence of kaempferol derivatives in blackberries,[1,7,24] but no kaempferol glycosides were detected in the genotypes we analysed.

The hydrophilic antioxidant capacities of crude berry extracts and the flavonol fractions isolated from the six blackberry genotypes are shown in Table 3. The hydrophilic antioxidant capacities of crude extracts isolated from the six blackberry genotypes ranged from 49.4 to 76.1 mmol TE kg^{-1} for $ORAC_{FL}$ and from 13.6 to 21.1 mmol TE kg^{-1} for PCL. Navaho had the highest total $ORAC_{FL}$ value (76.1 mmol TE kg^{-1}), followed by Apache (65.6 mmol TE kg^{-1}), Arapaho (61.9 mmol TE kg^{-1}), Kiowa (58.3 mmol TE kg^{-1}), Chickasaw (52.8 mmol TE kg^{-1}) and Prime-Jim® (49.4 mmol TE kg^{-1}). The $ORAC_{FL}$ values of the six blackberry genotypes were 8–36% lower than in our previous study (2002 crop), where $ORAC_{FL}$ values of the same six genotypes ranged from a low of 62.5 mmol TE kg^{-1} for Prime-Jim® (APF-12 in previous publication) to a high of 82.5 mmol TE kg^{-1} for Apache.[8] Since the genotypes were grown at the same location over both growing seasons (2002

Table 3. Hydrophilic antioxidant capacities (mmol TE kg^{-1} fresh weight) of blackberry genotypes

Genotype	Total $ORAC_{FL}$[a*]	$ORAC_{FL}$[b*]	Total PCL[c*]	PCL[d*]
Apache	65.6b	2.1b	21.1a	0.7a
Prime-Jim®	49.4f	2.3a	13.6d	0.6b
Arapaho	61.9c	1.5d	17.2b	0.5c
Chickasaw	52.8e	1.5d	16.3bc	0.5c
Kiowa	58.3d	1.8c	14.4cd	0.5c
Navaho	76.1a	1.8c	18.4b	0.5c

[a] Total $ORAC_{FL}$: oxygen radical-absorbing capacity of crude extract.
[b] $ORAC_{FL}$: oxygen radical-absorbing capacity of fractionated flavonol extract.
[c] Total PCL: photochemiluminescence assay of crude extract.
[d] PCL: photochemiluminescence assay of fractionated flavonol extract.
* Values within a column with similar letters are not significantly different (LSD, $P > 0.05$).

and 2003), it appeared that environmental growing conditions impacted the antioxidant capacity of the genotypes. The $ORAC_{FL}$ values for blackberries are in close agreement with those of Wu *et al*[32] (mean 52.5 mmol TE kg^{-1}, $n = 4$).

In contrast to the $ORAC_{FL}$ results, the crude extract of Apache (21.1 mmol TE kg^{-1}) had the highest antioxidant activity in scavenging superoxide anion radicals among the six blackberry genotypes, followed by Navaho (18.4 mmol TE kg^{-1}), Arapaho (17.2 mmol TE kg^{-1}), Chickasaw (16.3 mmol TE kg^{-1}), Kiowa (14.4 mmol TE kg^{-1}) and Prime-Jim® (13.6 mmol TE kg^{-1}). Inhibition of peroxyl and superoxide anion radicals by the flavonol fractions ranged from 1.5 to 2.3 mmol TE kg^{-1} and from 0.5 to 0.7 mmol TE kg^{-1} respectively for the blackberry genotypes, accounting for less than 5% of the total antioxidant activity against the two radical species. These results are consistent with total flavonols determined by HPLC (expressed as rutin equivalents) accounting for less than 5% of the total soluble phenolics determined by the Folin–Ciocalteu assay (expressed as gallic acid equivalents). The $ORAC_{FL}$ and PCL values from crude extracts showed moderate correlations with total phenolic content ($r_{xy} = 0.75$ and 0.88 respectively; see Table 6), while the two antioxidant measurements showed a less linear relationship ($r_{xy} = 0.70$), indicating that the phenolic compounds in different blackberry genotypes varied in their capacity to scavenge peroxyl and superoxide anion radicals. Since anthocyanins are the major contributors to $ORAC_{FL}$ in blackberries,[8] the differences in peroxyl and superoxide anion radical scavenging capacities most likely reflect compositional differences in anthocyanins among the genotypes.

The fractionated flavonols also showed moderate correlations with $ORAC_{FL}$ ($r_{xy} = 0.70$) and PCL ($r_{xy} = 0.77$) (see Table 6), and the two measurements showed a similar degree of correlation ($r_{xy} = 0.75$). These results indicate that the flavonols present in blackberry genotypes varied in their capacity to scavenge peroxyl and superoxide anion radicals. The moderate correlations observed between fractionated flavonols and $ORAC_{FL}$ and PCL values were unexpected, but may reflect differences in flavonol composition among the genotypes.

Flavonol components, total phenolics and antioxidant activities in blueberries

The contents of individual flavonols, total flavonols and total phenolics of blueberry genotypes are presented in Table 4. Total flavonol contents varied widely from a low of 192 mg kg^{-1} for US-720 to a high of 320 mg kg^{-1} for A-98, while Ozarkblue (234 mg kg^{-1}), Bluecrop (225 mg kg^{-1}) and US-729 (227 mg kg^{-1}) contained similar levels of total flavonols. The abundance of total flavonols and rankings of the five genotypes were similar to those in our previous study (2002 crop), where total flavonols ranged from a low of 173 mg kg^{-1} for US-720 to a high of 328 mg kg^{-1} for A-98.[8] The total flavonol content of Bluecrop (225 mg kg^{-1}) was slightly lower than our previously reported value (262 mg kg^{-1}),[8] but much lower than the value of 401 mg kg^{-1} reported by Skrede *et al*.[33] The levels of total flavonols in our study were much higher than the values previously

Table 4. Flavonol and total phenolic contents (mg kg^{-1} fresh weight[a]) of blueberry genotypes

	Genotype				
Compound	A-98[b]	Bluecrop	Ozarkblue	US-720[b]	US-729[b]
Myricetin 3-hexoside	40.9 ± 5.3[e]	13.2 ± 1.0	7.4 ± 0.5	27.9 ± 0.7	14.7 ± 0.6
Quercetin 3-rutinoside	8.9 ± 2.3	19.0 ± 0.5	11.1 ± 0.8	14.8 ± 0.8	7.3 ± 0.4
Quercetin 3-galactoside	150.0 ± 17.6	75.3 ± 2.6	42.1 ± 4.8	66.2 ± 5.4	61.7 ± 2.4
Quercetin 3-methoxyhexoside	15.5 ± 1.4	7.4 ± 0.5	4.8 ± 1.0	ND	10.8 ± 0.4
Quercetin 3-glucoside	18.3 ± 2.3	31.4 ± 1.6	24.9 ± 1.4	13.2 ± 0.7	13.8 ± 0.7
Quercetin 3-pentoside	ND	ND	5.8 ± 0.4	15.2 ± 0.9	30.8 ± 1.1
Quercetin 3-glucuronide	22.9 ± 2.6	16.0 ± 0.4	ND	ND	ND
Quercetin 3-glucosylpentoside	17.7 ± 2.3	12.1 ± 0.5	8.6 ± 0.9	12.0 ± 1.0	13.5 ± 0.4
Quercetin 3-caffeoylgalactoside	ND	11.7 ± 2.5	ND	ND	ND
Quercetin 3-caffeoylglucoside	ND	ND	7.6 ± 0.5	ND	ND
Quercetin 3-oxalylpentoside	ND	ND	31.4 ± 2.5	ND	ND
Quercetin 3-rhamnoside	13.9 ± 0.8	12.6 ± 3.3	58.1 ± 5.2	15.8 ± 1.2	52.4 ± 1.8
Quercetin 3-dimethoxyrhamnoside	ND	5.2 ± 1.7	4.7 ± 0.3	11.7 ± 1.2	ND
Quercetin 3-acetylgalactoside	16.6 ± 2.0	18.2 ± 1.3	24.8 ± 2.0	11.1 ± 0.4	6.6 ± 0.6
Quercetin 3-acetylglucoside	14.8 ± 1.7	3.1 ± 0.1	3.1 ± 0.2	ND	3.9 ± 0.1
Unknown[c]	ND	ND	ND	4.5 ± 0.5	11.0 ± 1.1
Total flavonols[d]	320a	225bc	234b	192c	227bc
Total phenolics[d]	3587a	2269d	2544c	3699a	3239b

[a] Data expressed as rutin equivalents for flavonols and gallic acid equivalents for total phenolics.
[b] Breeding selection not available for sale or present in commerce at the time of writing.
[c] Data expressed as rutin equivalents.
[d] Values within a row with similar letters are not significantly different (LSD, $P > 0.05$).
[e] Standard deviation ($n = 3$). ND, not detected.

reported by Bilyk and Sapers[1] (mean 26 mg kg^{-1}, $n = 4$), Häkkinen *et al*[34] (mean 45 mg kg^{-1}, $n = 2$), Häkkinen and Törrönen[35] (mean 48 mg kg^{-1}, $n = 5$) and Sellappan *et al*[5] (mean 181 mg kg^{-1}, $n = 5$). The TPH content of the blueberry genotypes ranged from a low of 2269 mg kg^{-1} for Bluecrop to a high of 3699 mg kg^{-1} for US-720. The contents of total phenolics fell within the wide range of values (1710–8680 mg kg^{-1}) reported in the literature for *V corymbosum* L cultivars and hybrids.[5,6,28,36] Similar to results obtained with blackberries, the levels of total flavonols determined by HPLC (expressed as rutin equivalents) and total phenolics determined by the Folin–Ciocalteu assay (expressed as gallic acid equivalents) did not correlate well ($r_{xy} = 0.20$), with the total flavonols accounting for only 5.2–9.9% of the total soluble phenolics in the blueberry genotypes.

The flavonols in blueberry were predominately quercetin derivatives. Quercetin 3-galactoside was the predominant flavonol in A-98, Bluecrop, US-720 and US-729, while quercetin 3-rhamnoside was the predominant flavonol in both Ozarkblue and US-729. Low levels of myricetin 3-hexoside were present in all genotypes except A-98 (40.9 mg kg^{-1}) and US-720 (27.9 mg kg^{-1}), where they were present in 2- to 4-fold higher concentrations. Quercetin 3-pentoside was present only in Ozarkblue (5.8 mg kg^{-1}), US-720 (15.2 mg kg^{-1}) and US-729 (30.8 mg kg^{-1}), while quercetin 3-glucuronide was found only in A-98 (22.9 mg kg^{-1}) and Bluecrop (16.0 mg kg^{-1}). Ozarkblue was the only genotype that contained quercetin 3-caffeoylglucoside and quercetin 3-oxalylpentoside. Quercetin glycosides accounted for >75% of total flavonols in the blueberry genotypes. Our observation is similar to a previous study, which reported quercetin as the predominant flavonol in southern highbush blueberries, followed by myricetin and kaempferol.[5] However, kaempferol was not detected in any of the genotypes tested in our study, which confirmed our previous finding[8] and other studies on *V corymbosum* blueberries.[1,34,35]

The hydrophilic antioxidant capacities of crude extracts isolated from the five blueberry genotypes ranged from 36.7 to 77.6 mmol TE kg^{-1} for $ORAC_{FL}$ and from 16.0 to 38.4 for PCL (Table 5). US-720 had the highest $ORAC_{FL}$ value (77.6 mmol TE kg^{-1}), followed by A-98 (71.4 mmol TE kg^{-1}), US-729 (64.1 mmol TE kg^{-1}), Ozarkblue (44.4 mmol TE kg^{-1}) and Bluecrop (36.7 mmol TE kg^{-1}). The $ORAC_{FL}$ values for blueberries are in close agreement with those of Wu *et al*[32] (mean 61.8 mmol TE kg^{-1}, $n = 8$) for cultivated blueberries.

Different trends in superoxide anion radical-scavenging capacity were observed among the blueberry genotypes. A-98 had the highest PCL value (38.4 mmol TE kg^{-1}), followed by US-720 (28.0 mmol TE kg^{-1}), US-729 (21.8 mmol TE kg^{-1}), Ozarkblue (19.1 mmol TE kg^{-1}) and Bluecrop (16.0 mmol TE kg^{-1}). The exceptionally high

Table 5. Hydrophilic antioxidant capacities (mmol TE kg^{-1} fresh weight) of blueberry genotypes

Genotype	Total $ORAC_{FL}$[a]*	$ORAC_{FL}$[b]*	Total PCL[c]*	PCL[d]*
A-98[e]	71.4b	5.2a	38.4a	1.4a
Bluecrop	36.7e	2.9e	16.0d	0.8c
Ozarkblue	44.4d	3.6d	19.1cd	0.9b
US-720[e]	77.6a	4.4c	28.0b	0.9b
US-729[e]	64.1c	4.8b	21.8c	0.9b

[a] Total $ORAC_{FL}$: oxygen radical-absorbing capacity of crude extract.
[b] $ORAC_{FL}$: oxygen radical-absorbing capacity of fractionated flavonol extract.
[c] Total PCL: photochemiluminescence assay of crude extract.
[d] PCL: photochemiluminescence assay of fractionated flavonol extract.
[e] Breeding selection not available for sale or present in commerce at the time of writing.
* Values within a column with similar letters are not significantly different (LSD, $P > 0.05$).

$ORAC_{FL}$ and PCL values for the small-fruited genotypes A-98 and US-720 were consistent with the high levels of total phenolics in the crude extracts. As previously noted,[4,37] small-fruited berries have a much larger surface area of skin relative to pulp and hence contain more anthocyanins, flavonols and $ORAC_{FL}$ on a per kilogram basis, since the polyphenolics are located predominantly in the skin.

Among all genotypes a linear relationship was observed between total phenolics and $ORAC_{FL}$ ($r_{xy} = 0.99$), while a less linear relationship was observed between total phenolics and PCL ($r_{xy} = 0.83$) (Table 6). Only a moderate correlation was observed between the two assays ($r_{xy} = 0.79$), indicating that various classes of phenolics in the crude extracts varied in their ability to scavenge peroxyl and superoxide anion radicals.

The peroxyl and superoxide radical-scavenging capacities of the flavonol fractions obtained from the five blueberry genotypes ranged from 2.9 to 5.2 mmol TE kg^{-1} and from 0.8 to 1.4 mmol TE kg^{-1} respectively, accounting for less than 5 and 8% of the total $ORAC_{FL}$ and PCL values respectively. These results are consistent with total flavonols accounting for less than 100 mg g^{-1} of the total soluble phenolics. The flavonols in blueberries correlated highly with PCL values ($r_{xy} = 0.89$) but showed a low correlation with $ORAC_{FL}$ values ($r_{xy} = 0.48$) (Table 6). The low

Table 6. Pearson's correlation coefficients[a] between $ORAC_{FL}$, PCL and total phenolics in crude extracts and between $ORAC_{FL}$ and PCL of flavonol fraction and total flavonols

	Total phenolics		Flavonol fraction	
	$ORAC_{FL}$[b]	PCL[c]	$ORAC_{FL}$	PCL
Blackberries	0.75*	0.88*	0.70^{ns}	0.77*
Blueberries	0.99**	0.83*	0.48^{ns}	0.89*

[a] The superscripts *, ** and ^{ns} denote significance at $P < 0.1$, significance at $P < 0.01$ and non-significance respectively.
[b] $ORAC_{FL}$: oxygen radical-absorbing capacity.
[c] PCL: photochemiluminescence assay.

correlation between flavonols and $ORAC_{FL}$ values suggests that compositional differences or complex interactions between individual flavonol glycosides can markedly impact peroxyl radical-scavenging capacity. Pinelo *et al*[38] reported that a mixture of catechin, resveratrol and quercetin acted in an antagonistic manner in comparison with the antioxidant capacity of the individual components. They also suggested that a specific polyphenol could exert a dominant role in influencing the antioxidant capacity trend of a mixture of polyphenolics and that a positive effect on overall antioxidant activity is not always expected by adding an additional flavonoid to a complex polyphenolic matrix. Although it is possible to isolate and measure the antioxidant activity of individual flavonol glycosides, it would be impractical to determine all the potential interactions owing to the large number of flavonol glycosides present in the fruit.

The two antioxidant measurements had a moderate correlation of $r_{xy} = 0.72$. Similar to results obtained with blackberries, blueberry flavonols varied in their capacity to scavenge peroxyl and superoxide anion radicals. This may be due to differences in flavonol composition among the genotypes and most likely reflects structural differences and synergistic/antagonistic effects among the individual flavonols present in the fractions.

CONCLUSION

Near baseline separation of berry flavonols by RP-HPLC using an Aqua® C_{18} column was achieved. The method allowed for the quantification of individual as well as total flavonols in different genotypes of blackberries and blueberries. Total flavonols accounted for less than 5 and 10% of the total soluble phenolics and antioxidant capacity in blackberries and blueberries respectively. Flavonol glycoside fractions derived from fresh blackberries and blueberries exhibited antioxidant activities against peroxyl and superoxide anion radicals, but the flavonols varied in their scavenging potential against the two radicals. Although different genotypes of blueberries and blackberries varied greatly in total flavonol content and antioxidant potential, the contribution of flavonols to total phenolics and total radical-scavenging capacity of the fruit was minor.

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REFERENCES

1 Bilyk A and Sapers GM, Varietal differences in the quercetin, kaempferol, and myricetin contents of highbush blueberry, cranberry, and thornless blackberry fruits. *J Agric Food Chem* **34**:585–588 (1986).
2 Heinonen IM, Meyer AS and Frankel EN, Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *J Agric Food Chem* **46**:4107–4112 (1998).
3 Wang SY and Jiao H, Scavenging capacity of berry crop on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J Agric Food Chem* **48**:5677–5684 (2000).
4 Kalt W, Ryan DA, Duy JC, Prior RL, Ehlenfeldt MK and Kloet SPV, Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp). *J Agric Food Chem* **49**:4761–4767 (2001).
5 Sellappan S, Akoh CC and Krewer G, Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *J Agric Food Chem* **50**:2432–2438 (2002).
6 Howard LR, Clark JR and Brownmiller C, Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *J Sci Food Agric* **83**:1238–1247 (2003).
7 Siriwoharn T and Wrolstad RE, Polyphenolic composition of Marion and Evergreen blackberries. *J Food Sci* **69**:233–240 (2004).
8 Cho M, Howard L, Prior RL and Clark JR, Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry, and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry. *J Sci Food Agric* **84**:1771–1782 (2004).
9 Prior RL, Lazarus SA, Cao G, Muccitelli H and Hammerstone JF, Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium Spp*) using high-performance liquid chromatography/mass spectrometry. *J Agric Food Chem* **49**:1270–1276 (2001).
10 Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Giampaoli S, Jansen A, Menotti A and Nedeljkovic S, Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. *Arch Internal Med* **155**:381–386 (1995).
11 Wedge DE, Meepagala KM, Magee JB, Smith SH, Huang G and Larcom LL, Anticarcinogenic activity of strawberry, blueberry, and raspberry extracts to breast and cervical cancer cells. *J Medicinal Food* **4**:49–51 (2001).
12 Hollman PCH and Katan MB, Health effects and bioavailability of dietary flavonols. *Free Rad Res* **31**:75–80 (1999).
13 Prior RL, Fruits and vegetables in the prevention of cellular oxidative damage. *Am J Clin Nutr* **78**:570–578 (2003).
14 Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, Hampsch-Woodill M, Huang D, Ou B and Jacob R, Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples. *J Agric Food Chem* **51**:3273–3279 (2003).
15 Popov IN and Lewin G, Antioxidative homeostasis: characterization by means of chemiluminescent technique. *Methods Enzymol* **300**:437–456 (1999).
16 Schlesier K, Harwat M, Böhm V and Bitsch R, Assessment of antioxidant activity by using different *in vitro* methods. *Free Rad Res* **36**:177–187 (2002).
17 Kalt W, McDonald JE, Ricker RD and Lu Z, Anthocyanin content and profile within and among blueberry species. *Can J Plant Sci* **79**:617–623 (1999).
18 Schieber A, Keller P and Carle R, Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography. *J Chromatogr A* **910**:265–273 (2001).
19 Schieber A, Hilt P, Conrad J, Beifuss U and Carle R, Elution order of quercetin glycosides from apple pomace extracts on a new HPLC stationary phase with hydrophilic endcapping. *J Separat Sci* **25**:361–364 (2002).
20 Slinkard K and Singleton VL, Total phenol analysis: automation and comparison with manual methods. *Am J Enol Vitic* **28**:49–55 (1977).
21 *User's Manual Photochem®*, 5th edn. Analytik Jena, Jena (2002).
22 SAS, *SAS/STAT Software: Changes and Enhancements, Release 8.1*. SAS Institute, Cary, NC (2000).

23 Wada L and Ou B, Antioxidant activity and phenolic content of Oregon caneberries. *J Agric Food Chem* **50**:3495–3500 (2002).
24 Henning W, Phenolics of fruit. XIV. Flavonol glycosides of strawberries (*Fragaria* × *ananassa Duch*), raspberries (*Rubus idaeus L*) and blackberries (*Rubus fruticosus L*). *Z Lebensm Untersuch Forsch* **173**:180–187 (1981).
25 Wald B, Galensa R, Herrmann K, Grotjahn L and Wray V, Quercetin 3-*O*-[6″-(3-hydroxy-3-methylglutaroyl)-β-galactoside] from blackberries. *Phytochemistry* **25**:2904–2905 (1986).
26 Kader K, Rovel B, Girardin M and Metche M, Fractionation and identification of the phenolic compounds of highbush blueberries (*Vaccinium corymbosum L*). *J Sci Food Agric* **55**:35–40 (1996).
27 Zheng W and Wang SY, Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J Agric Food Chem* **51**:502–509 (2003).
28 Moyer RA, Hummer KE, Finn CE, Frei B and Wrolstad RE, Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium. Rubus*, and *Ribes*. *J Agric Food Chem* **50**:519–525 (2002).
29 Wang SY and Lin HS, Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J Agric Food Chem* **48**:140–146 (2000).
30 Singleton VL, Orthofer R and Lamuela-Raventos RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods Enzymol* **299**:152–178 (1999).
31 Jaakola L, Maatta K, Pirttila AM, Torronen R, Karenlampi S and Hohtola A, Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. *Plant Physiol* **130**:729–739 (2002).
32 Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE and Prior RL, Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J Agric Food Chem* **52**:4026–4037 (2004).
33 Skrede GRE, Wrolstad RE and Durst RW, Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum L*). *J Food Sci* **65**:357–363 (2000).
34 Häkkinen SH, Karenlampi SO, Heinonen IM, Mykkänen HM and Törrönen AR, Contents of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *J Agric Food Chem* **47**:2274–2279 (1999).
35 Häkkinen SH and Törrönen AR, Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. *Food Res Int* **33**:517–524 (2000).
36 Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien B, Lischner N, Ehlenfeldt M, Kalt W, Krewer G and Mainland CM, Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium* species. *J Agric Food Chem* **46**:2686–2693 (1998).
37 Gao L and Mazza G, Quantification and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J Food Sci* **59**:1057–1059 (1994).
38 Pinelo M, Manzocco L, Nuñez MJ and Nicoli MC, Interaction among phenols in food fortification: negative synergism on antioxidant capacity. *J Agric Food Chem* **52**:1177–1180 (2004).